PROJECT NUMBER : 1904
PROJECT TITLE : Tobacco Physiology and Biochemistry
PROJECT LEADER : D. J. Ayers
WRITTEN BY : S. Wahab
PERIOD COVERED : December, 1990

I. LOW NICOTINE STUDY

A. **Objective:** To investigate the biochemistry of the nicotine biosynthetic pathway at the putrescine N-methyltransferase (PMT) step and specifically to isolate PMT from tobacco root extracts.

B. **Results:** The roots from Group 27 hydroponically grown, Burley 21 tobacco plants were harvested. These plants were very small in height and leaf size. The average root weight in this group of plants was also low compared to previous groups. Batches from these roots are being processed to determine PMT specific activity in this harvest (1,2,3).

Preparation of antibodies against PMT protein was initiated by isolating the 60 kD protein band from SDS-PAGE gels. PMT protein bands were sent to a contract laboratory for antibody production (4,5).

Several DEAE/AHS columns were used to process previously prepared phenyl-Sepharose fractions containing PMT enzymes. Good enzyme activity was obtained from some of these fractions (1,2,3,4). In addition, several previously prepared phenyl-Sepharose PMT samples were tested for protease activity. Two different temperature experiments (37°C & 4°C) were performed to assay for protease activity in these samples along with protein control. None of the Phenyl-Sepharose samples showed any protease activity (3).

A protein of interest to Miller Brewing (MP100) and bovine serum albumin (BSA) were successfully electroeluted from SDS-PAGE in sufficient quantities for cyanogen bromide (CNBr) digestion. Digestion of the purified proteins with CNBr showed distinct peptide fragments. The digestion of the partially purified MP100 extract revealed the same pattern of CNBr generated peptides as the SDS-PAGE purified MP100 (6). A PMT sample (4) was used for preliminary CNBr digestion. PMT was electrotransferred to an Immobilon-P filter and then digested with CNBr while still attached to the Immobilon-P. Four peptide fragments were visualized but at very low concentrations.

New preparations of poly(A+) RNA from mature tobacco roots were run on an agarose-formaldehyde gel. Northern blots were prepared by blotting the RNA from the agarose-formaldehyde gels onto nitrocellulose membranes (S&S). Fragments of approximately 700 and 400 bp, isolated from tobacco overly expressed clone pR17, were radioactively labeled and used as DNA probes to hybridize to total and poly(A+) RNA northern blots. A distinct band of more than 1,000 bp long was visualized due to hybridization of these fragments to total and poly(A+) RNA. This indicates that poly(A+) preparations are suitable for oligonucleotide hybridization

studies. Radioactive labeling of five oligonucleotides, derived from the putative PMT protein sequence, was initiated (7).

Several pGEM DNA standards were run on the AB1 automated DNA Sequencer to establish conditions for using Bio-Spin columns. In addition, DNA was prepared from several tobacco root clones to be used for sequencing (7,8).

Amplification of the putative PMT gene was attempted using three putative PMT primers and oligo dT via the polymerase chain reaction (PCR) method. A cDNA first strand was prepared from total and poly(A+) RNA. Amplification of this first cDNA strand has also been attempted by PCR (9).

C. Plans: Continue to prepare partially purified PMT from phenyl-Sepharose fractions through DEAE/AHS columns for CNBr digestion. Continue to process the newly harvested tobacco roots. Continue to develop a high radioactive labeling method for putative PMT synthetic oligonucleotides and start northern blot hybridization with these oligos. Attempt to amplify PMT sequences using PCR technology and clone a tobacco root cDNA fragment into a plant vector. Attempt to sequence several tobacco root clones.

D. References:

1. Lyle, J. Notebook No. 8856, p. 193.

2. Turner, D. Notebook No. 8973, p. 165.

3. Davies, S. Notebook No. 8976, p. 159.

4. Nakatani, H. Notebook No. 8384, p. 162.

5. Yu, T. Notebook No. 9002, p. 61.

6. Bower, P. Notebook No. 9032.

7. Wahab, S. Notebook No. 8983, pp. 139-141.

8. Michalik, T. Notebook No. 9036, p. 25.

9. Malik, V. Notebook No. 8974, p. 64.